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THE ETIOLOGY OF TRACHOMA

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Although many attempts have been made to determine the etiology of trachoma, it was not until recently that a germ has been demonstrated that could be considered specific. The early work was devoted largely to bacterial examinations, and such bacteria as Staphylococci, Streptococci, Pneumococci, Xerose bacilli, influenza bacilli, and the Diplococci of Morax-Axenfeld were found in the diseased tissue. None of these forms, however, was finally considered specific, and for a time the search was given up.

With the demonstration by Schaudinn of *Spirochaete pallida* as the cause of syphilis, a new impetus was given to the study, and it was not long before a body was demonstrated that was considered as the cause of trachoma. The discovery was made independently by Halberstaeder and v. Prowazek, and by Greeff.

While the bodies demonstrated have been pretty generally accepted as the cause of the disease there are still many problems to be solved. There is a difference of opinion as to what part of the mass described is the individual germ. Whether the germ is a bacterium or protozoon is another disputed point. Then there still remains the life history to be worked out in detail.

This disease seems to be closely related in its etiological factors to a number of other organisms supposed to be associated with certain other diseases. These include *Cytoryctes variolae* (small pox) *Cytoryctes vaccinia* (cow pox), *Neuroryctes hydrophobiae* (hydrophobia), the supposed organisms of scarlet fever, *Molluscum contagiosum*, chicken pest, carp pox, jaundice of the caterpillar, *Lyssa*, etc.

Halberstaeder and v. Prowazek, and Greeff claim the distinction of having first discovered and recognized the trachoma bodies. For a time the controversy ran high, each apparently describing different bodies, but now there is hardly any doubt that these investigators were dealing with the same organisms.

The technique is of very great importance in this study, and especially so the staining, and it was due to the difficulties in this particular that the early investigators failed to detect the organism. Adequate preparations may be obtained by making smears of the secretion, by lightly scraping the surface of the infected eyelids or sectioning the gross tissue removed by an operation. One great trouble is the presence of other organisms, as there is usually a mixed infection, and considerable extraneous matter such as pus cells and leucocytes.

Various methods of staining have been tried, mostly giving negative results. However the tubercular bacillus method, Löffler's stain for flagella, and the Giemsa method appear to give uniformly positive results. Greeff reports that Heidenhain's iron alum haematoxylin stain would not show the bodies, but this is the stain Leber and Hartmann use successfully and I have found it to work well.

The stain most frequently used in this work is Giemsa's azur-eosin. This is the stain used by Halberstaeder and v. Prowazek, and Greeff, Frosch, and Clausen in their work. The staining by this method is as follows:

1. 12 parts Giemsa's eosin solution (2.5 cc. of 1% French eosin solution to 500 cc. of distilled water).
2. 3 parts of Azur I (1:1000).
3. 3 parts of Azur II (0.8:1,000).

The three solutions must be well mixed. Then the preparation should be put in the mixture and kept at a temperature of 37° C. for 6-9 hours, then washed with distilled water, dried with filter paper and mounted in cedar oil.

In material from fresh cases of trachoma and from experimental cases on Orang Otangs, using the method of staining given above, v. Prowazek describes his results as follows: Surrounding the nuclei of the epithelial cells are dark amorphous masses (plastin masses). By careful differentiation and the use of colored light, there may be seen inside of these masses little red or reddish purple, round bodies. (Figure 1). These color after the manner of the nucleolus.

These trachoma bodies (the name was suggested by Greeff) have an average size of about $\frac{1}{4}$ micron, but they vary from the

limits of visibility to 1 micron, and it is possible that there are many beyond the limits of vision. They are seen to multiply by splitting and then come to lie two and two, resembling the arrangement of the Diplococci. In its usual position the plastin mass surrounds the nucleus like a cap, but as the little bodies increase rapidly in number, the plastin mass becomes puffed out, then it splits into little parts which become absorbed, and the little bodies are found near the nucleus, and these later make their way out of the cell.

The presence of the amorphous masses and the small bodies is typical of trachoma, and the morphological characteristics are specific. Halberstaeder and v. Prowazek, Greeff, Frosch and Clausen, and most of the men who have worked on trachoma, consider the small round bodies as the etiological originators (Erreger) of the disease, while they consider the dark amorphous mass as a reaction product of the cell of the host. As v. Prowazek describes it: The virus seizes on the host and after a short time becomes localized in the cell, where it reproduces. The host cell answers to the invasion of the virus by the production of a specific, morphologically differentiated substance of the cell, which is closely related to the nuclear substance or more closely to the nucleolus of the cell. These reaction products are usually found in the ectoderm cells.

There is a difference of opinion as to whether the small round bodies or the amorphous masses are the germs. Calkins, Lowden, Williams and Negri look upon the amorphous masses as protozoa, and the primary cause of the disease. Their work however has been done on other diseases and they explain the conditions of trachoma by analogy. On the other hand the men who have done work on trachoma, including Halberstaeder, v. Prowazek, Greeff, Frosch, Clausen, Hartmann, Leber, Di Santo, consider the small round bodies as specific germs, and the amorphous masses as reaction products.

The authors who see in the amorphous masses the germ of the disease consider the whole mass as an individual protozoon. There is considerable constancy in the appearance of these forms, which would easily lead to this conception, but in old cases of the disease they are usually absent, while the small bodies may be present. In support of this theory they describe a uninucleate cell, but on this

point they are not very definite. It is to be supposed that the whole mass is considered the nucleus. This mass is so structureless that it hardly appears probable that it is the nucleus of an organism. It has also been claimed, though it has never been confirmed, that conjugation has been observed; also the formation of cysts. They interpret the small bodies as being idiochromidia, and forming part of the life history of the organism. This leads to their classification as rhizopods, in which group the formation of idiochromidia is characteristic. The work that led to these conclusions was done on other forms than trachoma, and at present the proof is not very conclusive that the same cycle is followed in the case of the trachoma organisms.

v. Prowazek gives the following reasons for taking exceptions to the foregoing views: (1) In vaccinia the masses can be made to disappear with a 10-20% solution of NaCl, and yet vaccination can be successfully performed with the material that is left. The same results can be obtained after twenty-four hours digestion with trypsin or pepsin. (2) In vaccinia successful vaccination can be performed with material in which no Guarneri bodies are microscopically visible. (3) In hydrophobia the Negri bodies are often not visible in virulent material. (4) Infections can be successfully performed with weakened emulsions of hydrophobia virus that have been treated centrifugally, while in the liquid thus treated no Negri bodies were visible, having apparently been cast aside. (5) Finally, the structure of these bodies speak against their being protozoa, as they possess no protoplasmic structure, are hyalin, fairly homogeneous, and subject to changes that would be considered cell degeneration processes rather than stages in the development of a protozoan.

While the criticisms are significant, it must be remarked that the infectiousness of the material in which no bodies were visible only shows that it is likely that there only remained the smaller bodies which we infer are ultra-microscopic. It is true that it is easier to conceive of the presence of the smaller bodies which in their largest and visible stage are only 1 micron in size, as ultra-microscopic, but this does not preclude the possibility of the larger bodies existing in this state, especially after treatment.

The fact that when treated centrifugally the virus still retained its virulence might possibly be explained by the fact that some of the bodies are so light that the centrifuge had no effect on them, or perhaps some of the virus might have stuck to the side of the container. This seems probable when it is considered that in chicken pest it has been found that a solution of 1-1,000,000,000 of the virus retains its virulence.

Now to take up the contention that the small bodies are the germs of the disease. In vaccinia of the cornea of the rabbit, v. Prowazek was able to trace in the epithelial cells and less frequently in the Guarneri bodies themselves, little alveoles in which were minute oval or round bodies, which he designates as initial bodies. Division was described in some of these at times as binary and in other cases as multiple spore formation. The same bodies were also seen by Hartmann, Paschen and Mühlert and considered by them as the organisms of the virus.

Paschen found in vaccinated children many vaccine bodies in the lymph of the pustule, and besides these many that divided binarily, remaining connected by the ends, and these again divided forming a chain. These finally separated forming little round bodies with a thread like flagellum hanging to them.

Babes found similar bodies in hydrophobia, while Negri and Velpine could differentiate little nuclear like bodies in the Negri bodies and their alveoles. These lay arranged symmetrically around a central body that could not be differentiated. v. Prowazek suggests that they probably came from this by multiple division.

So here in these other diseases we find a condition somewhat similar to that found in trachoma. Thus we are able to interpretate trachoma as caused by a related etiological factor.

According to v. Prowazek, trachoma is a disease of the epithelium. On the introduction of the virus into the eye, the epithelial cells of the conjunctive enlarge. This growth continues with the increase of the virus, and finally the cell is ruptured and the germs spread out over the pus. After this the disease can be spread by purely mechanical means, through contact with the pus cells. By the bursting of the cells the surface layers come to be covered with the germs. A few little bodies may then reach the follicles, a

condition which is usually considered as the primary stage of the disease but which according to this author is the secondary result due to rapid growth. In the follicles one also finds epithelial cells, and consequently the bodies may be found there, as it is in the epithelial cells that they are primarily localized. It is only in the old cases that the free bodies are found, probably due to the cells containing them having been destroyed.

Leber and Hartmann, staining with Heidenhaim's iron alum haematoxylin, show a light court or ring surrounding the trachoma bodies which is colored by the Giemsa stain. (Figure 2). This light ring is interpreted as cytoplasm, while the dark body is considered as the nucleus. They describe division as follows: The dark body in the centre divides forming two small bodies, but these move a short distance apart, remaining connected by a thread. In moving apart the light surrounding mass (cytoplasm?) is pulled along, and the result is a dumb bell shaped figure. (Figure 3.) These figures are very similar to those of *Babesia* of the tick fever in the dividing stage. These authors claim that after the entrance of the trachoma bodies into the cell, chromatin material is thrown out, and this changes to plastin, and while it is not probable that all the plastin material is formed from chromatin, at least a part of it is. This formation is typical of cell degeneration, as has lately been shown by R. Hertwig, Hartmann, and Reichow working on other forms.

The trachoma bodies increase rapidly in number until they come to fill up the entire space of the cytoplasm of the cell. (Figure 4)) This increase in the number of the trachoma bodies has a destructive effect on the cell. The nucleus is forced to the edge of the cell (Figure 5) and finally destroyed (Figure 6). In this way the cell is destroyed and after its destruction the bodies come to lie free, and may get into the pus or on the surface of the conjunctiva.

There seems to be little doubt that the bodies described are the germs of the disease, and the amorphous masses reaction products of the cell. It might be added that the trachoma bodies have been described in the nucleus (Figure 2) and lying free in the cell in the early stages, apparently before the reaction product had been formed. In the destruction of the cells and the irritation caused

by these bodies, there is ample explanation of the ravages of the disease. Besides this when we consider that in all fresh cases, the presence of the bodies is constant, and that their morphology is always the same, we add one more point in favor of this view. Furthermore these bodies have never been found in any other form of eye disease. All forms of conjunctivitis and infections of the eyes have been examined, but the results have all been negative.

A point that has caused a great deal of doubt is that after filtration through the finest Berkefeld filter under pressure, the filtrate was found to be infectious, although no bodies were visible. The explanation of this phenomenon is that the smaller ultra-microscopic bodies passed through the filter, but this is purely hypothetical. Prowazek, working on chicken pest, which presents the same problem, has been able to clear up the matter. He has shown that by using a Berkefeld filter and filling the pores with agar, celloidin and gelatin that the filtrate was no longer infectious. In the gelatinous mass he found the broken bodies of the germs.

In cases that have been treated and in old established cases no trachoma bodies have been found. Greeff has shown in at least one case that at a certain stage the disease is not infectious, but unfortunately he does not state whether or not the trachoma bodies were present. The indication seems to be that there is a stage in the life cycle of the germ when it is not present in the form described. Another fact suggestive of the life history, is that the disease is recurrent (recidiv). After an apparent cure that has lasted some time, the disease suddenly comes back in full vigor, when there was no apparent new infection. This is a very important consideration in the matter of the suppression of the disease, and probably accounts for the great number of cases that slip into this country despite the care of the United States Public Health and Marine Hospital Service. In this respect it reminds us of the action of malaria, and it is interesting to note that in 1897 Elze published a paper trying to show the relation of trachoma to the *Plasmodium* of malaria.

Many experiments have been made to grow the germ in culture. The secretion has been planted in: (1) Different fertile soils; (2) on bouillon; (3) on agar, weak and strong; (4) on glycerin

agar; (5) on blood agar; (6) on Löffler's blood serum; (7) on serum agar; (8) on soils free from acids. The contents of follicles were also planted in the soils. On no occasion were the trachoma bodies obtained. This does not mean that they cannot be grown in culture, but rather that the right conditions have not been tried.

Although only negative results were obtained in the attempts to grow the germ in culture, they have been grown in culture animals. Halberstaeder and v. Prowazek succeeded in infecting Orang Otangs and obtaining the typical trachoma bodies. Positive results were not obtained on all occasions, even on repetition; this was so both when the secretion and the follicles themselves were used for the inoculation. There is a possibility that the virus was not all retained, but was washed away by the lachrimation. There is also a possibility that some of the animals were naturally immune, and also that the cases from which the virus was taken were not in an infectious stage of the disease.

Inneculation experiments have been made on other animals, both for the purpose of attempting to get material for experimentation and also for hygienic considerations. Experiments have been made to inoculate rabbits, guinea pigs and dogs, but with negative results. However Greeff remarks that he remembers seeing a dog that apparently had trachoma, the eyes presenting all the clinical symptoms; and Dr. Kunz reports a dog kept in the trachoma barracks at Thorn afflicted with the disease. In both of these instances however, no microscopical examinations were made to find the trachoma bodies.

Greeff was unable to infect the *Macacus* apes, while the Italian investigators, Baiardi and Bertarelli, and Cecchetto obtained positive results both on the *Macacus* and *Cercopithecus*. The time that elapsed before it became certain that the infection was successful varied from two weeks to three months. The fact that these investigators working in Italy were able to get successful inoculations, while Greeff in Germany was unsuccessful with the same species, suggested that climatic conditions may have something to do with the infectiousness of the virus.

Successful inoculation were performed on the *Cynocephalus* (Pavinae) apes by Hess and Römer, Greeff, and Dr. Hereford.

The latter stated that after a time the symptoms began to disappear and five weeks after the inoculation had disappeared entirely. This suggests that the disease may run in a cycle, or that the culture animal had a high resistance.

A fact that seems pertinent in regard to these experiments on animals is that even in cases where it was apparent that the infection had taken, the condition of the conjunctiva was different from that in man. The inflammation was not so great and there were no infected follicles present in the manner so characteristic in human cases. Greeff remarks that "while the conjunctiva reacts to the trachoma virus, the condition of the susceptibility and in the structure of the clinical pictures there is established a great difference between man and animals. Perhaps it is the same as we have learned in the case of the syphilis virus, that the animals give a lesser susceptibility as against a greater in man."

Greeff has performed two inoculation experiments on humans. In the first case no results were obtained. There could be no doubt that the person from whom the virus was taken had the disease. Two possibilities present themselves to explain the failure of the experiment:—either that the person on whom it was made was inherently immune to trachoma, or that the period of infectiousness had been run. Greeff offers the latter explanation. It is the general opinion of clinicians that the disease has its periods of infectiousness, and after this has been run that it is no longer infectious. That the virus from the eye of this patient of Greeff's experiment was at one time infectious seems certain, as two members of his family had apparently caught it from him previously.

In the second case the results were distinct. On the second day after the inoculation there was a reddening, swelling and secretion of the left eye, but the difference between the two eyes was slight. Five days later there was a swelling of the lid, slight swelling of the conjunctiva, and a slight secretion, the two eyes being well differentiated. Three days later there was a distinct swelling of the lid and conjunctiva with the formation of follicles. The next day the eye was running, the lids heavily swollen, the conjunctiva very red, and folds and follicles formed, presenting a typical trachoma picture. The microscopical examinations showed no cell inclusions

until the thirteenth day, when the first were visible. After this they increased rapidly.

From these results we can draw the following conclusions: Trachoma is a specific infectious disease; it is transmissible by purely mechanical means, without the presence of an intermediate host; there is no acute or initial stage of the disease, differentiated from a secondary or tertiary stage as in the case of syphilis since trachoma develops completely in a few days. The fact that with the introduction of the virus the trachoma bodies, cell inclusions, are brought into the conjunctiva, which before was free from these, speaks strongly for the conception of these bodies as the specific germ of the disease.

The question as to susceptibility and immunity to this disease has often been raised. Is there any such thing as disposition of a race as a whole to this disease? The only fact seeming to have any bearing on this question is that the negroes in North America seem to be immune from it. As far as is known no case has ever been reported among them. On the other hand in Europe and Asia it is found to a great extent among the colored races, and in Africa it is very prevalent, Egypt seeming to have been its original home. On the whole it may be said that race has no proved significance in this connection.

It is interesting to note that children up to the age of two or three years seem to be immune. At least no cases have been noted in very young children, even when they have been suckled by mothers having the disease.

It has been stated that persons having gonorrheal infections of the eyes are immune, but this is not so. It has also been said that scrofula prepares the eye for trachoma infection, but there is no proof of this. Of course a person in this condition may be highly susceptible as there is a favorable condition for the retention of the virus, if brought into contact with the eye.

It has been contended that when one eye is infected the other becomes immune. It is true that it is usually found only in one eye, but the reason probably is that after one eye becomes infected the patient becomes more careful and keeps the other eye clean. Personally I have seen a case in which both eyes were infected.

Germaix tried infecting the free eye with the virus of the other, but was unsuccessful. This however does not prove anything as it is possible that he took the virus from an eye in which the infectious stage had been run.

People whose blood contains a large amount of haemoglobin are more likely to escape the disease. Anemia and the presence of CO₂ are favorable for the disease.

In the lowlands and swampy regions the disease is especially prevalent, while in the highlands it is less often found. This is due in a great measure to the conditions found in these regions. In the swampy countries there is a greater amount of CO₂ which is favorable to the disease, and dampness is also a favorable condition. In the highlands the conditions are less favorable for the disease as the people are found to have more haemoglobin in the blood and less CO₂ as a rule.

It is probable that climatic conditions influence the virility, the re-occurrence and the infectiousness of the disease. While it is not found exclusively in any one place, it has been longest and best known in Egypt, a warm country. It is also noticeable in the clinics that the disease is less frequent in winter, and that with the return of the warm weather, new infections or re-occurrences come.

There can be little doubt that trachoma is a germ disease, or that the germ is present in the morphological structures described. Our knowledge of the subject points to the so-called trachoma bodies as the germ, rather than the larger amorphous masses. The point then remains, *is the parasite a protozoon or bacterium?*

Halberstaeder and v. Prowazek claim that the parasites are protozoans, while Greeff, Fresch and Clausen claim that they are bacteria. In support of the theory that they are protozoans are the following facts: (1) The disease and also the related diseases run in cycles so characteristic of protozoan diseases. It has been noticed that there is a re-occurrence after an apparent cure without a new infection. (2) The trachoma bodies are intracellular. The only cases of bacteria that are intracellular are the leper bacilli described by Babes, but this is strongly opposed by Sudakowitsch; and the Gonococci, whose intracellular nature is now explained as due to the action of phagocytes. (3) They have also been found in the nucleus,

a condition that has never been observed in the case of any bacteria. (4) They give certain reaction products that can be traced morphologically, and by staining, to the stainable matter of the nucleus (chromatin and nuclein). (5) They can not be grown in culture media as can the bacteria. (6) They are very adaptive, changing according to the environment. (7) They give new qualities to their host. (8) Gall and gall salts, which have no effect on bacteria, destroy their virulence. (9) The division as pictured by Leber and Hartmann, shows a condition analogous to that in forms of protozoa, especially as observed in amoeba and flagellates. (10) They are similar morphologically to forms of protozoa found parasitic in amoeba. (Figure 7). (11) They are smaller than any known bacteria.

Greeff, who considers them as bacteria, says that the fact that they have not yet been grown in culture does not prove that they are not bacteria, but that the right medium has not been found. Further he argues that the smallness of the body is no criterion as Frosch has shown some bacteria equally as small. Unfortunately it is not stated what these bacteria are. Another point that he emphasizes is that the double form is characteristic of the bacteria, but with binary fission this form would be seen in any case.

I think that the evidence now at hand greatly favors the contention that the body is a protozoon. There still remains the question of their classification, but with our present meagre knowledge of the subject little can be said. They are undoubtedly closely related to the germs of the other diseases that were mentioned as being closely related to trachoma. Prowazek creates a new group to put these forms in, calling them Chlamydozoa, i. e., mantle bearers (referring to the fact that they surround the nucleus in the form of a cap or mantle) and placing this group midway between the bacteria and protozoa.

What can be said as to the practical application of the trachoma bodies in the diagnosis of trachoma? In new cases that have never been under treatment the presence of these bodies is constant and specific for this disease, but in most cases there is little need to make so complicated an examination as the clinical picture is typical. In old cases, or cases that have been under treatment, the bodies

disappear, and then while the patient might be suffering from trachoma, this form of diagnosis would be contradictory. It may be said that this method is only confirmatory and not sufficient in itself. When the results are positive there can be no doubt that trachoma exists, but when these bodies are absent the disease may still exist.

Through the kindness of Dr. A. Barkan, in charge of the eye clinic of the Cooper Medical College, I was enabled to obtain material from trachoma patients. This material was used to demonstrate the etiological factor of the disease and to experiment on the susceptibility of animals. The work was done in the laboratory of the Department of Zoology of the University of California, with the following results.

Material was obtained from the eye of a man, forty-five years of age, who claimed that his eyes had never before been treated. Smears of the secretion of the eye were made, the contents of follicles were spread upon cover glasses, and sections were made from the conjunctiva obtained from an operation. These preparations were stained with the Giemsa stain and some with Heidenhain's iron alum haematoxylin.

Examination of the preparations stained by the Giemsa method showed many pus cells and leucocytes present in the sectioned material. Many small eosinophile granules were also present. In the parts of the sections showing but few pus cells or leucocytes the trachoma bodies were found. These, as described above, were minute deeply staining bodies, lying in a structureless amorphous mass. These were occasionally found surrounding the nucleus, but most often they were to be seen lying free outside of the cells in rows, with the amorphous masses broken up and persisting only occasionally. These bodies showed also in the other preparations and with the iron alum haematoxylin stain.

Other material was obtained from the eye of a Chinaman from the secretion and by opening follicles. It was found, however, impossible to demonstrate any trachoma bodies. In this case it was discovered that the patient had been under treatment before, and as has been stated above, in such a case the bodies do not seem to be present. The question however arises as to what has become of the bodies. When the patient was brought into the clinic, it ap-

peared that the disease was in a virulent condition, and apparently rapidly getting worse. Now if these bodies are the direct cause of the disease why should they be absent in such a condition. The answer may be given that they may be present and in ultra-microscopic condition, or that while the body of the germ has been destroyed a toxin has remained. These explanations, however are purely theoretical, and more information is necessary before we can give a definite explanation.

Attempts were made to infect guinea pigs, white rats, and a dog, with the disease. The virus for these experiments was obtained from a patient with trachoma who at the time that the virus was obtained had never been under treatment for her eyes. The results were all negative. The eyes of the inoculated animals showed no clinical symptoms of the disease and the microscopical examinations were also negative.

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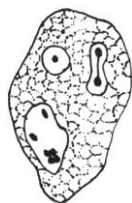
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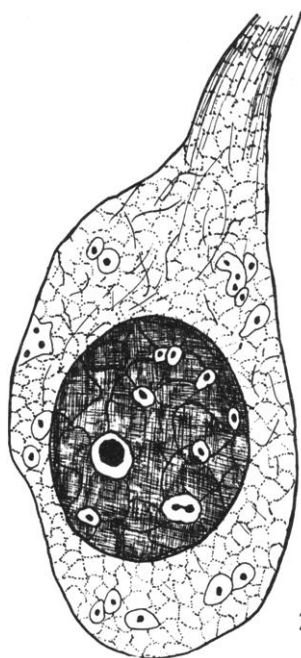
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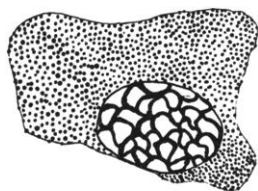
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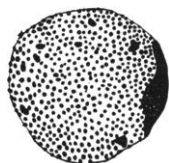
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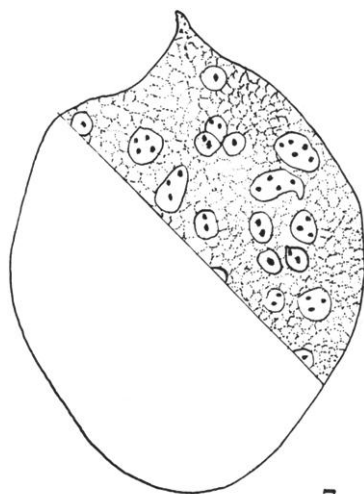
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PLATE VI

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EXPLANATION OF FIGURES

Fig. 1. An epithelial cell with a few trachoma bodies surrounding the nucleus.

Fig. 2. Trachoma bodies surrounded by light court as figured by Leber.

Fig. 3. Division of trachoma bodies as figured by Leber.

Fig. 4. Cell in which trachoma bodies have greatly increased in number.

Fig. 5. Nucleus being crowded to edge of cell.

Fig. 6. The destruction of the cell, the nucleus having disappeared.

Fig. 7. An Ameba infected with protozoan parasites. (After Calkins).